AD 296 479

6.3-2-4

Final Report No.1 *REPORT ON*

CONTRACT NO DA 92-557-FEC-36609

INCLUSIVE DATES 15 Oct. 1961 *TO* 14 Oct. 1962

SUBJECT OF INVESTIGATION

HISTOCHEMICAL STUDIES ON THE DISTRIBUTION OF ENZYMES, ESPECIALLY OXIDASES AND PHOSPHATASES IN THE LIVING BODY

RESPONSIBLE INVESTIGATOR

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Office of the Chief of Research and Development
United States Army
APO 343

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Final Report No. 2

[redacted]

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Histochemical studies on the distribution of enzymes, especially oxidases and phosphatases in the living body

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ABSTRACT

Comparative study of cytochemical reactions in the blood cells, cornea and the neuroglia was made on 44 different vertebrates. Among the vertebrates examined, the peroxidase in the leucocytes was evenly distributed, while the phosphatase in the leucocytes was found sporadically. The strongest reaction of peroxidase in leucocytes was found in man, while that of alkaline phosphatase in leucocyte was in lower animals such as guinea pig and salamanders. The most interesting phenomenon in the comparative hematology was that the peroxidase reaction of eosinophil leucocyte was not recognized in Felidae, Trichocephali, and nor in Anoura despite that all other animals higher than crocodile possessed the reaction positive eosinophil leucocytes in blood. This phenomenon was interpreted to be a phylogenetical gap in the evolution of blood cells. The significance of this phenomenon probably lies in the complicated mechanism of blood formation. Again, from the standpoint of comparative hematology, the phosphatase reaction was less significant than the peroxidase reaction because the former reaction varied greatly in intensities according to the methods employed.

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Contents

I. Introduction

The phosphatases esterases are responsible for the breakdown of [illegible], while the oxidases including peroxidases belong to the respiratory enzymes in tissues. These two different enzyms are quite important for metabolism in the living body.

The alkaline phosphatase reaction is generally detected in the kidney, intestine, liver, bony tissue, capillary wall, skin including sweat gland, retina, cornea, and others. The peroxidase reaction is generally detected in the myeloid granular leukocytes, Kupffer cells, endothelial cells lining the sinusoids of liver, capillary wall, and the germ follicles.

In 1952 we devised a simple peroxidase staining method using benzidine and diethyl ammonium sulfate and named this NAS-Benzidine reaction. Further, in 1953, while we were carrying out an investigation into [illegible] of the histochemical peroxidase reaction, it was confirmed that o-phenylenediamine can be used in place of benzidine for staining of peroxidase in leukocytes. By these technics an [illegible] finding was found in which case the results of the cytochemical reaction are varied according to the substrate used, further [illegible] is quite sensitive to one substrate, while not at all to another substrate. Accordingly we should always consider these characteristics in cytochemical reaction when comparing reaction intensities among various animal cells.

The present study primarily deals with distribution of peroxidase,

alkaline phosphatase, and P.A.S. reactive substance in the blood cells, cornea and the neuroglia of various animals from the standpoint of comparative anatomy.

II. Materials and methods.

For material 44 different animals were utilized. They include 11 species of mammals, 3 species of birds, 3 species of reptiles, 6 species of anura, 14 species of urodela and 7 species of fishes. The detailed names of the species are shown in Tables 1-4.

The blood was teken from the heart, subcutaneous vein, or from the tail and smear proparations were made. For the study of the neuroglia and cornea, some of the above-mentioned animals were chosen and the necessary parts of the body were carefully extracted.

In the study of tissue culture of neuroglia, cerebrum and cerebellum of a 7 day old rabbit, just new-born kitten or 14 day chicken embryos were cultured for about 2 weeks by the roller tube method. The fluid nutrient medium used in these culture consists of 50% Gey's [illegible] salt solution, 45% human ascitic fluid and 5% embryo [illegible] (8 day chicken embryos). The medium contains also [illegible] glucose and 1000 units per ml. of penicillin.

The peroxidase reaction wsn employed as follows:

a. Fixation. Fix with ethanol-formol (9 : 1 [illegible] minute (blood smear), then wash in distilled wa[illegible].

b. Immerse in benzidine-hydrogen peroxide solution (Mitsui et al. 1951) for 5 minutes, or in orthophenylenediamine-hydrogen peroxide solution (Mitsui et al. 1955) for 5 minutes.

c. Wash in running water.

d. Counterstain with dilute Giemsa stain.

The alkaline phosphatase reaction was employed as follows:

a. Fixation. The blood smear was fixed with gaseous formaldehyde for 10 minutes and then let stand in the air for over 30 minutes. The tissue was fixed with cold acetone for one hour, then washed in distilled water.

b. Incubate in the following substrate solution at 37° C for 10 hours.

Sodium β-glycerophosphate	0.5 gr.
Glycocoll-natron buffer (pH 9.3)	20 cc.
Distilled water	20 cc.
0.75% $CaCl_2$	50 cc.
5% $MgCl_2$	10 cc.

c. Wash in the buffer solution above mentioned for 1-2 minutes, then wash in distilled water only for a moment.

d. Immerse in 2% lead acetate solution at 37°C for 30 minutes, then wash in distilled water.

The periodic acid Schiff (P.A.S.) reaction for polysaccharide, Sudan black-B staining for lipid, the Azan staining for connective tissue fiber, nucleic acid staining, and the [illegible] were employed as usual.

II. Results obtained

1. Peroxidase and alkaline phosphatase reactions in the leukocytes.

(1) Peroxidase reaction.

The comparison of the peroxidase reaction intensity is based on the shortest time necessary for the development of the peroxidase reacting granules of blood cells. For instance, the shortest time is 5-10 seconds in the benzidine reaction and 30 seconds in the orthophenylenediamine reaction in human leukocytes.

a) Neutrophil leukocytes.

In general, the peroxidase reaction of neutrophil leukocyte is the most intense in man.

The next intense group :
is hamster (rodent), salamander (urodele amphibian), toad, lizard (reptile), bull frog, Rana nigromaculata (anuran amphibian), horse and dog.

The moderate group :
is pig, cat, rabbit, monkey, mouse, albino rat and cow.

The weak group :
is tortoise (reptile) and most fishes.

The negative group :
is bird. It should be noted, however, that the avian leukocyte comparable to the human neutrophil leukocyte is pseudoeosinophil leukocyte.

b) Eosinophil leukocytes.

The most intense group is man just like the case of neutrophil leucocyte.

The next intense group is hamster, toad, bull frog, lizard, striped snake, dog, Rana nigromaculata, horse, and domestic fowl.

The moderate group is rabbit, cow, monkey, pig, black rat, and duck.

The weak group is guinea pig, mouse, albino rat, trotoise and sparrow.

The negative group is cat (Felidae) in mammal, viper (Trigonocephali) in reptile and most species of urodela (see Table 2) and all the fishes.

As to the peroxidase reaction of eosinophil leucocytes there were two important points.

The first point was that a profound phylogenetical gap can be found in Felidae (cat) and viper.

The second was that reaction disappears at some species of urodela (Table 2) and never appears in lower animals than the urodela, namely in most of urodela, and fishes (Table 3). In other words, a boundary line between peroxidase positive and negative groups can be drawn within the urodela (Table 2).

There were no reaction negative eosinophil

leucocytes in anura although both anura and urodela belong to the same amphibian group (Table 1).

(2) Alkaline phosphatase reaction.

The blood film fixed with gaseous formalin was incubated in the substrate solution for 10 hours. This incubation time was common in all the animals examined, so the reaction intensities were compared with the volume, density or color of the reaction endproduct deposited in the leukocytes. The eosinophil leukocyte is generally reaction negative when glycerophosphate is used for the phosphatase substrate. Therefore, only the neutrophil leukocytes of various animals were compared in the present study.

The alkaline phosphatase activity of neutrophil leukocyte is found in cytoplasm, but not in nucleus. In submammalian group there are relatively few animals whose neutrophil leukocytes indicate alkaline phosphatase activity. Even in mammalian group, however, Japanese macaque, dog, and mouse did not show any reaction activity (Table 4).

In general, the alkaline phosphatase reaction was strong in horse, guinea pig, hamster, Amphiuma means tridactylum, and giant salamander.

The weak group was rabbit, albino rat, cattle, pig, man, lizard, tortoise, toad, and copper rockfish. It is of

interest that human neutrophil leukocyte indicated very weak alkaline phosphatase reaction despite that the leukocyte indicated the strongest peroxidase reaction among all the animals.

The negative group was Japanese macaque, dog, mouse, birds, snake, frog, most of the salamanders and of the fishes examined.

Generally speaking, the alkaline phosphatase activity of the neutrophil leukocytes becomes weaker as the evolutionary degree of animal becomes lower.

The comparison of alkaline phosphatase and peroxidase reactions of various animal leukocytes is demonstrated in Table 4.

2. Some cytochemical reactions of the neuroglia cells in tissue culture

In general, neuroglia cells in the central nervous tissue are morphologically classified into three kinds, astrocytes, oligodendroglia and microglia, by application of the silver impregnation technique.
However, the biological characteristics of these neuroglia cells which are morphologically differentiated from each other are yet almost unknown. This is the reason that the subject of the neuroglia tissue moves towards the biological investigation of neuroglia

cells. In correlation with modern cytological techniques such as tissue culture or electron microscopic study, it is natural to consider the application of cytochemical methods for the purpose of the biological study of neuroglia cells. In this investigation, we have attempted the cytochemical observations of neuroglia cells, especially astrocytes in tissue culture.

a) Alkaline phosphatase.

The granules in the cytoplasm, nucleolus, coarse granules in nucleoplasm of astrocytes show positive activity. The nucleoplasm itself and cytoplasm of astrocytes do not show, in general, any activity. Also in the cytoplasmic processes of astrocytes, alkaline phosphatase positive granules are seen. The nucleolus shows strong activity.

b) Periodic acid-Shiff reaction(PAS.).

In the cytoplasm of astrocytes PAS.-positive granules are commonly found. In the cytoplasm of some cells, PAS.-positive materials appear the cloud-like mass, stained intensively. These PAS.-positive granules are present also in the processes of the cytoplasm, sometimes accumulate at one side of the cytoplasm or around the nucleus. The protoplasmic astrocytes which have widely expanded protoplasmic membrane contain

much less PAS-positive granules in some cells scarcely any. The nucleus is also weakly positive. The macrophages show strong positive PAS-reaction as a whole of the cell body.

c) Fatty substance.

In general, the cytoplasm of astrocytes contains a large amount of fatty substance which is stained black and granular by Sudan black B. These granules entirely fill up the cytoplasm of some cells. The size and quantities of fatty granules are variable. In general, the granules of membranous astrocytes are smaller and fewer, and are diffusely scattered in the cytoplasm, corresponding with the Golgi area and a loss of granules is seen in some cells. Sudan black B stained granules of small size are present in the processus of the cytoplasm. In the nucleoplasm, fatty granules stained by Sudan black B are seldom found.

The comparison of the alkaline phosphatase reaction, P.A.S. staining and of fat among animals is shown in Table 6.

b) Nucleic acid.

The granules in the nucleus of astrocytes, which are stained red by the Feulgen-Rossenbeck's nucleic acid staining appear to be chromatin granules, presumably containing desoxyribonucleic acid.

By the Korson's method, the nucleoplasm of astrocytes is weakly and diffusely stained greenish. The nucleolus and cytoplasm are stained weakly violet-oranged colored. As results of these reactions, the existence of desoxy-ribonucleic acid(DNA) and ribonucleic acid(RNA) are presumed also present in the astrocytes in tissue culture. By the methylgreen-pyronin staining for RNA and DNA, in most cells which are seen in the cultured materials, their cytoplasm is stained reddish by pyronin and nucleoli are more intensively red. In the nucleoplasm a few methylgreen positive fine granules are found.

e) Glycogen.

Glycogen is negative in the astrocytes.

f) Proteins. (Mercuric bromphenol blue reaction)

On the astrocytes, the nucleolus shows the most strongest bluish reaction and the nuclear membrane a faint reaction. The nucleoplasm and cytoplasm are stained diffusely fine granular or homogenously. In some astrocytes, the cytoplasm reacts extensively in the perikaria around the nucleus. The oligodendrocytes show strong reaction as a whole of the cell body The processes of the cytoplasm, either in astrocytes or oligodendroglia, are scarcely stained or not at all.

3. Some cytochemical reactions of the cornea.

The eyeballs of monkey (Formosan macaque), Uroloncha domestica and of Rana nigromaculata were extracted to study cytochemical charateristics of the cornea for which alkaline phosphatase reaction, P.A.S. reaction, and Azan staining were carried out.

In a vertical section through the cornea, the following layers can be generally seen: 1) the epithelium, 2) the Bowman's membrane, 3) the stroma or substantia propria, 4) the Descemt's membrane, 5) the endothelium of the corneal mesenchymal epithelium.

The epithelium is stratified squamous, and it consists, as a rule, of three to five layers of cells. The alkaline phosphatase reaction occurred in this epithelium very intensely in monkey and frog, while relatively weakly in bird (Uroloncha domestica). In the epithelium the reacting granules of the alkaline phosphatase appeared within the cytoplasm, especially at the periphery of the cells, and also intercellular spaces. The granules could not be found in the nucleus. These findings were common in both monkey and frog. The epithelium in bird was stained more faintly than in monkey and frog. However, in general, the alkaline phosphatase reaction did not occur in the other layers than the epithelium. The substantia propria consists of regular connective tissue

fiber bundles which were intensely stained by the P.A.S. staining indicating a large amount of polysaccharide. These bundles were also stained deeply blue by the Azan staining which primarily reacts with the collagen fibers.

The Bowman's membrance and Descemet's membrane were not clearly seen in the animals as compared to the human cornea under hematoxylin eosin staining. But, when examined with the P.A.S. staining, these membranes, especially the Descement's membrane, could be recognized very distinctly. There is no doubt that the Bowman's membrane and the Descemet's membrane correspond to the basement membranes of the stratified epithelium and the endothelium respectively.

In the endothelium or the corneal mesenchymal epithelium which is a single layer of squamous cell covering the inner surface of the Descement's membrane, neither alkaline phosphatase reaction nor P.A.S. reaction occurred.

The comparison of the alkaline phosphatase reaction in each layer of the cornea among animals is shown in Table 5.

IV. Discussion.

The data from comparative hematology hitherto investigated lead to the conclusion that continuous evolution of blood cells can generally be admitted regarding the presence of nucleated erythrocytes and the shape as well as the number of

erythrocytes except in several species. On the other hand, it is well known that human leukocytes other than the lymphatic series demonstrate an intense peroxidase reaction in the absence of disease, and that the reactions of animal leukocytes are frequently of quite different nature.

In the present study, the activities of peroxidase and alkaline phosphatase reactions of animal blood cells were compared. It was made clear that human eosinophil and neutrophil leukocytes possess the most intense peroxidase activities among vertebrates, but that the alkaline phosphatase reaction of them is rather weak or entirely negative. As to the cytochemical reactions of animal blood cells, some interesting findings in comparative hematology were obtained.

It was reported by Tokue (1929) for the first time that the specific eosinophil granules of cat eosinophil leukocyte in the peripheral blood lack peroxidase activity. Nakamura (1955) and Mitsui et al. (1956), made comparative studies on the peroxidase reaction of blood cells in various animals, and stated that there were peroxidase negative eosinophil leukocytes also in the animals other than cat. In the present study, it was ascertained

that Felidae (cat) and Trigonocephali (vipor) possessed peroxidase negative eosinophil leukocytes, thet a few kinds of urodela still possessed peroxidase positive eosinophil leukocytes, and that all the fishes examined possessed no peroxidase positive eosinophil leukocyte. Recently Fey (1962) reported that Xenopus laevis Daudin, one of Australian frogs, has peroxidase negative eosinophil leukocyte, too. In other words, as far as the peroxidase reaction of the eosinophil leukcyte is concerned, a few phylogenetical gaps are found in mammals, reptiles and anura. The significance of the phylogenetical gaps mentioned above is not obvious. There may be some relationships between occurrence of the peroxidase negative eosinophil leukocytes and mechanism of the blood formation.

The neutrophil leukocytes in the blood of animals generally possess peroxidase activity although there are wide variations in the reaction intensities. The reaction negative leukocytes can be found only in the avian blood, and, as is well known, these avian leukocytes are called pseudoeosinophil leukocytes. Concerning the fish blood, the neutrophil leukocyte is peroxidase positive except for Selachii and herring. These findings

are quite different from those of eosinophil leukocytes which are peroxidase negative in all the fishes.

Regarding the distribution of alkaline phosphatase activity in the neutrophil leukocyte among vertebrates, a definite phylogenetical relationship was not recognized. Generally speaking, the neutrophil leukocytes with the alkaline phosphatase activity can be found more often in mammals than in submammalian groups, and the alkaline phosphatase in the neutrophil leukocytes is not evenly distributed in vertebrates from mammals to fishes. There are not a few neutrophil leukocytes without alkaline phosphatase activity in mammals, reptiles, and amphibia, whereas the peroxidase positive neutrophil leukocytes are evenly found in those animals. The avian neutrophil or pseudoeosinophil leukocytes essentially differ from those of mammals in that in the former neither peroxidase nor alkaline phosphatase reaction appears.

It is worthy of special comment that the results of the phosphatase reaction are frequently influenced by the cytochemical technics employed. It was already evidenced by Kato (1957) that the neutrophil leukocytes of some cold-blooded animals are essentially phosphatase positive while they change into phosphatase negative when counter-stained with dilute Giemsa stain. Further it is not

uncommon that hte data on the phosphatase reaction of the same animal is different according to investigators. This is probably due to the cytochemical method employed by them as mentioned above.

In general, the neuroglia includes ependyma which lines the ventricles of the brain and spinal cord, neuroglial cells, neuroglia fibers, and the satellite or capsular cells of the peripheral ganglia. The cell of Schwann of the peripheral nerves may be considered equivalent to peripheral neuroglia. There are three types in the neuroglia proper: astrocytes, oligodendroglia or oligodendrocytes, and microglia. The first two are called macroglia which are undoubtedly of ectodermal origin, as are the nerve cells proper. The third, or microglia, originates from mesodermal cells of the pia mater which migrate into the central nervous system along the blood vessels.

The astrocytes have abundant granular cytoplasm and numerous, rather thick plasmatic expansions or long, relatively thin, smooth, and little branched expansions. The oligodendroglia has smaller cytoplasm and nucleus than those of astrocytes, and the name is derived from the fact that their few and slender processes have few branches. The microglia has small, deeply stained

nucleus and scanty cytoplasm.

The microglia is scattered everywhere throughout the brain and spinal cord.

Despite these morphological differences, the differences of biological characters among them are not obvious sufficiently. The neuroglia appears to be an important mediator for the normal metabolism of the nervous elements proper, although little is known in this respect. In the present study, it was attempted to know cytochemical characters of these three types of neuroglia cells in tissue culture, further to differentiate these types by cytochemical reactions such as alkaline phosphatase reaction, P.A.S. reaction, fat staining and protein reaction. However, these three types of neuroglia cells did not show any cytochemical specific nature respectively, and yet no remarkable difference was found in animal species.

The protein reaction of neuroglia cell was already studied by Shimai et al. (1961) using the mercuric bromphenol blue (Hg-BPB) method which is considered to detect SH or COOH group. But, particular findings were not yet obtained by them.

Again the criteria for identification of types of macroglia in electron micrograph have been agreed upon.

As to the cytochemical reactions of the cornea, the stratified squamous epithelium generally has an intense alkaline phosphatase activity. However the epithelium of Uroloncha domestica showed relatively weak reaction activity. This weak reaction activity should be examined on many other kinds of bird by further investigation. The alkaline phosphatase reaction does not occur in the other parts of the cornea tissue, while the P.A.S. reaction distinctly occurs in the stroma, the Bowman's membrane and in the Descemet's membrane. It seems strange that the stroma containing many lymph vessels or capillaries, does not show any alkaline phosphatase activity because the endothelial cells covering a lumen of blood vessels frequent show a strong activity. It was already described by Maeda (1952), that the corneal epithelium of amphibians and fishes shows an intense alkaline phosphatase reaction and that the epithelium of reptiles shows a weak reaction.

It is admitted that the transparency of the cornea is high, though less than that of the aqueous humor and of glass. It is probably due to the regularity of its structural composition and also to other factors of chemical nature still incompletely understood. It will require more investigation.

V. Summary.

Comparative study of some cytochemical reactions in the blood cells, cornea, and the neuroglia in tissue culture was made on 44 different vertebrates from mammals through to fishes. The data from this comparative histochemical study are summarized as follows:

1. The peroxidase is distributed in the granular leukocytes evenly in vertebrates with very few exceptions. Man possesses the most intense peroxidase activity in both eosinophil and neutrophil leukocytes among the animals examined. Eosinophil leukocytes of cat, viper, and Xenopus lack peroxidase activity indicating a profound gap in phylogeny of animal blood cells. This peroxidase reacting substance in eosinophil leukocytes disappears in some class of urodela, and never appears in lower animals than these urodela. The approximate order of the peroxidase intensity of neutrophil leukocytes in vertebrate is as follows: man, anura, urodela, reptile, fish. Birds possess peroxidase negative pseudoeosinophil leukocyte which is comparable to human neutrophil leukocyte.

The alkaline phosphatase activity is not found in eosinophil leukocyte, while it is frequently found in cytoplasm of neutrophil leukocyte. The phosphatase activity of neutrophil leukocyte is intense in amammals except for monkey, dog, and mouse in which no activity is

evidenced. Generally speaking, however, this activity turns weaker as the evolutionary degree of animals becomes lower. Therefore the neutrophil leukocytes with the alkaline phosphatase activity are comparatively few in number or entirely lacking in submammalian group. Again, the alkaline phosphatase reaction is less significant for comparative hematology than the peroxidase reaction, because the result of the former reaction is frquently varied according to the method employed.

2. Cytochemical characteristics of the cultured central nervous tissue, especially neuroglia cells of rabbits, cats and chickembryos were investigated. In general, neuroglia cells do not show any cytochemical specific natures respectively. Therefore, as the result of these observations in tissue culture, we could not differentiate three kinds of neuroglia cells. The granules in the cytoplasm and cytoplasmic processes, nucleolus and coarse granules in nucleoplasm of astrocytes, show positive activity of alkaline phosphatase. In the cytoplasm of astrocytes, there are intensive PAS-positive granules. The macrophages show strong positive PAS-reaction as a whole of the cell body. Various kinds of cultured cells in the nervous tissue, especially, astrocytes and fibroblasts contain a large amount of fatty substance.

In some cells, a loss of fatty granules is seen corresponding

with the Golgi area. The existence of DNA and RNA is presumed in the cultured cells of the nervous tissue. The nucleolus of most cells in tissue culture shows intensive protein reaction. The cytoplasm and nucleoplasm are also reactive diffusely fine granularly or homogenously.

Comparatively, these kinds of granules which show positive activity of alkaline phosphatase, PAS, reaction and stainability by fat staining in the cytoplasm of astrocytes are considered to be much in common concerning the shape, varieties of size, localization, and the animal species.

3. A strong alkaline phosphatase activity was proved in the stratified squamous epithelium of the cornea in both higher and lower animals equally. However, in the cornea of bird the reaction activity of the epithelium was relatively weak. The alkaline phosphatase reaction did not occur in the endothelium lining the lymph canaliculi of the substantia propria, nor in the endothelium lining the anterior chamber of the eye ball. The substantia propria, the Bowman's membrane and the Descemet's membrane were all characterized by a strong P.A.S. reaction indicating the presence of a large amount of polysaccharide.

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Table 1 Peroxidase reaction of eosinophil leukocyte of anura

	Substrate I	Substrate II
Brown frog (Rana japonica)	+	+
Tree frog (Hyla arborea japonica)	+	+
Rana nigromaculata	+	+
Bull frog (Rana catesbiana)	+	+
Bufo vulgaris	+	+
Rana pipiens	+	+

Substrate I = Benzidine + H_2O_2

Substrate II = o-phenylene-diamine + H_2O_2

+ = Positive reaction

− = Negative reaction

Table 2 Peroxidase reaction of eosinophil leukocyte of urodela

	Substrate I	Substrate II
Triturus viridescens viridescens	+	+
Pseudotriton ruber	+	+
Triturus pyrrhogaster	–	–
Hynobius tokyoensis	–	–
Onychodactylus japonicus	–	–
Megalobatrachus japonicus	–	–
Necturus maculosus	–	–
Amphiuma means tridactylum	–	–
Triturus torosus torosus	–	–
Plethodon dunni	–	–
Ambystoma gracile	–	–
Ambystoma maculatum	–	–
Ambystoma tigrinum	–	–
Rhyacotriton olympicus	–	–

Table 3 Peroxidase reaction of eosinophil leukocyte of fishes

	Substrate I	Substrate II
Etrumeus micropus	—	—
Parapristipoma trilineatum	—	—
Copper rockfish (Sebastodes caurinus)	—	—
Hake (Merluccius productus)	—	—
Rat fish (Hydrolagus colliei)	—	—
Skate (Raja binoculata)	—	—
Dasyatis akajei	—	—

Table 4 Comparison between peroxidase and alkaline phosphatase reactions of neutrophil leukocytes

Animals		Peroxidase reaction	Alkaline phosphatase reaction
Mammals	Man	+	+
	Japanese macaque	+	—
	Cattle	+	+
	Horse	+	+
	Pig	+	+
	Dog	+	—
	Rabbit	+	+
	Guinea pig	+	+
	Albino rat	+	+
	Mouse	+	—
	Hamster	+	+
Birds	Domestic fowl	—	—
	Duck	—	—
	Urolonchа domestica	—	—
Reptiles	Tortoise (Clemmys japonicus)	+	+
	Lizard (Takydromus tocky-dromoides)	+	+
	Striped snake (Elaphe quadri-virgata quadrivirgata)	+	—
Anuran amphibians	Brown frog (Rana japonica)	+	—
	Rana nigromaculata	+	—
	Bufo vulgaris	+	+

Table 5 Alkaline phosphatase reaction of the cornea

	Epithelium	Substantia propria	Endothelium
Monkey	+++	—	—
Urolonchа domestica	+	—	—
Rana nigromaculata	+++	—	—

Table 6 Cytochemical reactions of neuroglia cells

	Alkaline phosphatase	P.A.S.	Fat
Rabbit	+	+	++
Cat	+	+	++
Fowl	+	+	++